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16. ABSTRACT

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Toxicological evaluation of complex industrial wastes: implications for exposure assessment*

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SUMMARY

We evaluated a variety of short-term bioassays to construct a battery of tests that could be used for assessing the biological effects of potentially hazardous complex industrial wastes. Ten samples were studied for hepatotoxicity; these samples and an additional 5 were studied for mutagenicity. Although the data are limited to these samples, the results suggest that the *Salmonella* assay (strain TA98) or a prophage-induction assay (both in the presence of S9) in combination with determination of relative liver weight and levels of a set of serum enzymes in rats may provide a battery of tests suitable to characterize complex industrial wastes for mutagenic and hepatotoxic potential. The biological activities exhibited by the wastes were not readily predicted by the chemical profiles of the wastes, emphasizing the importance of characterizing potentially hazardous complex industrial wastes by both chemical and biological means. DNA from liver, lung and bladder of rats exposed to some of the wastes was analyzed by the ^{32}P-postlabeling technique for the presence of DNA adducts. A waste that produced mutagenic urine produced a DNA adduct in bladder DNA. The implications of this approach for assessment of exposure to complex hazardous waste mixtures are discussed.

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INTRODUCTION

In the United States, wastes are classified as hazardous if they possess one of a number of physical characteristics or if they are composed of certain industrial process wastes or contain specifically regulated chemicals [1]. Guidelines for characterizing the biological toxicity of hazardous wastes have not yet been promulgated; however, knowledge of the potential biological toxicity of a hazardous waste could augment the available chemical characterization and provide relevant information regarding potential health effects.

Because hazardous wastes may contain a wide array of chemical mutagens, and because no single bioassay will detect all chemical classes of mutagens, several short-term assays with different genetic end-points may be advantageous for screening hazardous wastes for genotoxic potential. Consequently, combinations of short-term assays have been proposed as possible screening batteries for hazardous wastes [2]. Nonetheless, most investigations of the genotoxicity of hazardous wastes have not used a battery of assays. Instead, most studies have used only the *Salmonella* assay [3,4], although mammalian cells and other eukaryotic assays have been used to a limited extent [5–7].

In addition to genotoxicants, many hazardous wastes may also contain chemicals that are suspected or known hepatotoxicants. Hepatomegaly and abnormal liver function, with return to normal on cessation of exposure, have been associated with human exposure to hazardous waste [8–10]. The liver was a primary target organ in mice exposed subchronically to Love Canal soil [11]. Thus, we have evaluated the acute hepatotoxicity of 10 chemically characterized wastes and assessed whether the chemical analysis was predictive of the biological results.

This report summarizes the efforts of our laboratory to determine the usefulness of a variety of short-term bioassays for assessing the genotoxic and hepatotoxic potential of hazardous industrial wastes. Most of the wastes we evaluated had been partially chemically characterized, permitting comparisons of biological activity and chemical composition. The chemical analysis indicated that many of the wastes contained carcinogenic metals, chlorinated compounds, and solvents that are detected poorly by the *Salmonella* assay [12–14]. Thus, in addition to the *Salmonella* assay, we included a prophage-induction assay in *Escherichia coli* that may be more sensitive than *Salmonella* for these classes of compounds [15,16].

Because in vivo mammalian metabolism may be a critical factor in the generation of mutagenic metabolites from complex hazardous wastes, we studied the mutagenicity of urine from rats administered hazardous wastes by gavage. Because most batteries include mammalian cell assays, we evaluated a series of such assays and compared the results with those obtained with *Salmonella* for a set of 4 diverse hazardous wastes. The advantages and disadvantages of testing crude versus extracted waste samples are discussed along with some of the problems encountered when trying to select appropriate test methodologies for a wide variety of hazardous waste types.

TABLE I

PHYSICAL DESCRIPTION OF HAZARDOUS WASTES

Wastes	Description
Waste Set 1	
A	Black, thin oil
B	Black, oily liquid
C	Black pourable tar
D	Composite of aqueous wastes; watery liquid with oil drops
E	Composite of organic wastes; thin, dark liquid
F and G	Organic wastes; biphasic, thick gray sludge with reddish-brown liquid
H and I	Aqueous wastes; thin, gray slurry
J	Composite of organic wastes; gray, thick liquid with suspended solids
K	Similar to J, but lighter in color and thinner
L and M	Composite of organic wastes; black, thin, pourable tar
N and O	Composite of aqueous wastes; clear, watery liquid
Waste Set 2	
P	Light-brown liquid with suspended solids from coke plant
Q	Brown clear liquid from herbicide manufacturing plant
R	Brown semi-solid with wood chips from pulp and paper mill
S	Dark liquid with brown flocculant and oil drops from oil refining plant

Measuring the extent of exposure of populations to complex hazardous wastes is of vital importance. Thus, we explored the possible use of the recently developed ^{32}P-postlabeling procedure [17,18] to detect DNA adducts from tissues of rats exposed to two of the hazardous wastes. The implications of these results for exposure assessment are discussed.

MATERIALS AND METHODS

Wastes and waste extracts

Two sets of hazardous waste samples were used for these studies (Table I). The first set was obtained from Edward L. Katz, Hazardous Waste Engineering Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. Three of the waste samples (A, B, and C) were from 3 different industrial manufacturers. The remaining 12 samples (D through O) were from 3 commercial hazardous-waste incineration facilities that burn a mixture of hazardous wastes composited from a variety of industrial sources and from one incinerator whose waste source was not specified. Each waste sample was analyzed for the presence of a limited number of priority organics and/or metals identified in the U.S. EPA Appendix VIII list of priority pollutants (Table II) [19]. Dichloromethane (DCM) extracts were solvent-exchanged into dimethyl sulfoxide (DMSO), and crude wastes and waste extracts were tested in the *Salmonella* assay. Crude wastes were evaluated in the prophage-induction assay and

TABLE II

CONCENTRATION OF CHEMICALS AND METALS IDENTIFIED IN HAZARDOUS WASTES ($\mu g/g$)

Chemical/Metal[a]	Company 1	Company 2	Company 3	Hazardous waste incineration facilities											
				1		2				3		4			
				Aqueous	Organic	Organic		Aqueous		Organic		Organic		Aqueous[c]	
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Aniline		14000	550000												
Benzyl chloride	3000														
Bis-(2-ethyl-hexyl)-phthalate		500		< 100	3800							200	230		
Butylbenzyl-phthalate				< 100	320	120	450	< 5	8	160	140				
Chlordane												< 60	< 60		
Chlorophenylisocyanate		21000													
Cresol(s)										2000	2500				
Diethylphthalate						620	1300	240	240						
m-Dichlorobenzene		2300													
o-Dichlorobenzene		46000													
p-Dichlorobenzene		59000													
m-Dinitrobenzene			< 100												
Diphenylamine			6200												
2,4-Dimethylphenol										500	2000				
Hexachlorobutadiene												< 10	< 10		
Hexachloroethane	560														
Hexachlorocyclopentadiene												< 10	< 10		
Isophorone				< 100	110										
Mononitrobenzene			< 100												
Naphthalene	< 100			< 100	350	250	450	44	49	450	490	38	33		
Phenol				34000	1000	1500	1700	1800	2900						
Phenylenediamine			2300												
Phenylisocyanate		160000													
Trans-1,4-dichloro-2-butene	59000														
1,2,4-Trichlorobenzene		290													

Benzene				<3	260										
Carbon tetrachloride[b]	68000	44000		<2	6000	3700	4400	<10	<10	5900	5700	46000	58000	<10	<10
Chlorobenzene		4100										9100	11300	<10	<10
Chloromethane		1200										390	500	<10	<10
Chloroform	2900					170	270	21	22			110	60	<10	<10
cis- 1,4-Dichloro-2-butene	18000														
Methylene bromide												<10	<10	3100	4700
Methylene chloride	21000											110	340	44	60
Methyl ethyl ketone				27	9700					18000	38000				
Tetrachloroethylene	11000			<1	28	7100	9800	1600	1300	7900	8100	87	150	<10	<10
Toluene	240000			110	2400	32000	45300	2900	2700	56000	48000	160000	390000	22	20
1,1,1-Trichloroethane	<100									24000	16000	330	230	<10	<10
Trichloroethylene[b]	4000	40000		<1	5500	3700	4400	90	85	8100	7800	8300	10300	<10	<10
Ag	<3					<1	<1	<1	<1	<1	<1	<1	<1		
As	<24					<20	<20	<20	<20	<14	<14	<23	<23		
Ba	<7					110	140	6	7	1160	1150	990	1100		
Be	<2					<1	<1	<1	<1	<1	<1	<1	<1		
Cd	<5					6	6	<1	<1	153	15	49	55		
Cr	<5					50	57	3	3	431	425	250	290		
Hg	<22					<10	<10	<10	<10	<4	<4	<50	<50		
Ni	68					7	7	2	2	26	27	<4	<4		
Pb	<19					140	150	<10	11	1830	1800	1200	1300		
Sb	<12					58	61	<10	<10	437	373	<24	<24		
Se	<470					340	<100	<100	<100	<21	<21	<160	<160		
Tl	<23					<20	<20	<20	<20	<9	<9	<22	<22		
pH		2		8		7	7	6	7				7	7	7
Water(%)	2		5	94	3	38	48	95	97	21	23	5	5	94	96
Characterized mass (%)	45	39	61	97	6	43	54	96	98	33	36	27	52	94	96

[a]Data from Ref. [19].

[b]Waste samples B, E, F, G, J, K, L and M were spiked with these two chemicals.

[c]Previous versions of this Table [20, 23, 27] incorrectly showed values for wastes N and O for the following compounds: bis-(2-ethylhexyl)-phthalate, chlordane, hexachlorobutadiene, hexachlorocyclopentadiene, and naphthalene. Wastes N and O were not analyzed for the presence of these 5 compounds. Also, the values reported previously [20, 23] for Si for wastes J and K, should have been reported as values for Ag.

administered by gavage to rats to determine hepatotoxicity and to detect mutagenic urine [20].

The second set of hazardous wastes (Table I) consisted of 4 wastes from 4 different industries: coke plant, herbicide manufacturing, pulp and paper mill, and oil refining. These wastes were obtained from Dr. M. McKown, Battelle Columbus Laboratories, Columbus, OH. DCM extracts were prepared as described [21] and solvent exchanged into DMSO for bioassay. These 4 waste extracts were tested in *Salmonella* and in the mammalian cell assays.

Urine and urine extracts

Male F-344 rats were dosed with 4 different dosages of waste C for 10 days before collecting 24-h urine from 3 rats per dose. However, the available amounts of waste samples permitted the use of this protocol for only waste C. For 9 other wastes, a single dose of the crude waste was administered by gavage to male F-344 rats. All urines were collected on dry ice for 24 h, centrifuged, filter-sterilized, and frozen at −20°C. As reviewed previously [20], β-glucuronidase has generally been required to observe rodent urinary mutagenicity. Thus, all 10 raw urines were tested for mutagenicity in *Salmonella* TA98 with S9 and β-glucuronidase. In case the addition of β-glucuronidase to the plate was inadequate to identify mutagenic urine from rats exposed to the complex wastes, 6 of the raw urines were also extracted and tested as follows.

One ml of β-glucuronidase (Sigma Type VII from *E. coli*) at a concentration of 1000 units/ml of potassium phosphate buffer (0.15 M, pH 7.4) was added to 2–10 ml of thawed urine, and the mixtures were incubated with shaking for 1 h at 37°C. Then, each mixture was poured through two serially connected Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA) followed by water. Concentrates were then eluted with methanol, the methanol was evaporated, and the residue was solvent-exchanged into a volume of DMSO to produce 5× concentrates, which were stored at −20°C.

Salmonella assay

The *Salmonella* plate-incorporation assay was performed as described [22]. Wastes and waste extracts were assayed at least twice (on separate days), in duplicate, in strains TA98 and TA100 in the presence and absence of Aroclor 1254-induced Sprague-Dawley rat liver S9 (~1 mg of protein/plate) prepared as described [22]. Urines and urine extracts were assayed in strain TA98 in the presence of S9 and β-glucuronidase (1000 units/plate). Raw urine and urine extracts were tested twice (on separate days), each in singlet due to small sample volumes. A dose-related increase in the number of revertants/plate was considered a positive response.

Prophage-induction assay

The Microscreen prophage-induction assay developed by Rossman et al. [15] was

performed using modifications described previously [23]. The two bacterial strains used for this assay are derived from *E. coli* B/r. $WP2_s(\lambda)$ is a lambda lysogen of $WP2_S$ (*trpE*, *uvrA*); SR714 (*trpE*, $uvrD_3$) is the indicator strain. The lysogenic strain was exposed overnight to various dilutions of the crude wastes both in the presence and absence of S9. Following exposure, each suspension was sampled for the presence of lambda particles by plating onto the indicator strain. The criterion for a positive response was an increase in the number of induced plaque-forming units per plate that reached or exceeded the upper limit of the 99% confidence interval based on the negative controls.

Mammalian cell assays

Because of the limited amount of DCM extract of each of the 4 wastes used in the mammalian cell assays, and because of the cost of performing a set of such assays with each extract, it was not possible to perform the assays according to currently accepted protocols or established guidelines. Instead, limited protocols were used that required a minimum number of steps and amount of sample to permit an extract to be identified as a presumptive positive or negative. In order to conserve sample, the dose range for each extract was estimated for all of the assays by performing a preliminary cytotoxicity study in Chinese hamster ovary (CHO) cells. The assays were then performed once with only a few doses of extract and in the presence of S9. Otherwise, the assays were performed essentially as described below.

The L5178Y/$TK^{+/-}$ −3.7.2C mouse lymphoma assay was performed in the presence of S9 as described [24]. Cytogenetic effects induced by the 4 waste extracts in the presence of S9 were determined by scoring for chromosomal aberrations and sister chromatid exchanges (SCEs) in CHO-WBL cells as described [25]. The ability of the 4 waste extracts to induce morphological transformation in Balb/c-3T3 cells was determined as described [26]. Metabolic activation was provided by X-irradiated rat liver cells, and 12-*O*-tetradecanoyl-13-phorbol acetate (TPA) was used to promote the formation of the transformed phenotype.

Hepatotoxicity assays

Ten waste samples were evaluated for hepatotoxicity as described [27]. Briefly, male F-344 rats were exposed by gavage to a single dosage of waste that ranged from 0.5 to 5 ml/kg. 24 h after dosing, the rats were weighed, anesthetized with 50 mg/kg of sodium pentobarbital i.p., and exsanguinated from the abdominal aorta. Serum chemistry profiles were obtained for concentrations of total bilirubin (BILI) and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKPH), and lactate dehydrogenase (LDH). The activity of ornithine carbamyl transferase (OCT) was determined as described [27].

The liver was excised quickly, rinsed in saline, blotted, and weighed, and then relative liver weight (liver-to-body-weight ratio) was determined. Samples from the left lobe were taken for open histopathological examination; lesions were evaluated on

hematoxylin- and eosin-stained tissue sections. Data were analyzed as described by Simmons et al. [27].

DNA adduct analysis

^{32}P-ATP (3000 Ci/mmol, 10 mCi/ml of aqueous solution containing 5 mM 2-mercaptoethanol) was obtained from Amersham, Arlington Heights, IL. Polyethyleneimine (PEI) cellulose thin-layer chromatography plates were prepared as described previously [28] except that the PEI solution (50% aqueous) was obtained from Aldrich Chemical Co., Milwaukee, WI. Micrococcal nuclease and nuclease P1 were purchased from Sigma, St. Louis, MO; calf spleen phosphodiesterase was from Boehringer Mannheim, Indianapolis, IN; T4 polynucleotide kinase was from Pharmacia, Inc., Piscataway, NJ; and ^{32}P-labeled orthophosphate was purchased from Amersham. All other chemicals were of analytical grade.

Because of limited sample, one rat per treatment group was gavaged with waste M (5 ml/kg) or waste L (2.5 ml/kg) and then sacrificed 24 h following exposure. (These wastes were selected primarily because they produced mutagenic urine.) DNA

TABLE III

GENOTOXIC RESPONSES OF CRUDE WASTES/EXTRACTS AND RAW URINES/EXTRACTS

Wastes	Mutagenic responses in *Salmonella*										Prophage-induction responses	
	Crude wastes				Waste extracts				Urines[a]			
	TA98		TA100		TA98		TA100					
	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9	Raw	Ext.	+S9	−S9
C	−	−	+	+	+	−	+	−	+	+	+	+
L	+	−	−	−	−	−	−	−	+	+	+	+
M	+	−	−	−	−	−	−	−	+	+	+	+
G	+	+	−	−	−	−	−	−	−	−	+	+
O	+	−	−	−	−	−	−	−	−	−	−	−
E	−	−	−	−	−	−	−	−	−	−	NT[b]	NT
H	−	−	−	−	+	+	−	−	−	NT	+	+
J	−	−	−	−	+	−	−	−	−	NT	+	−
K	−	−	−	−	−	−	−	−	−	NT	−	−
B	−	−	−	−	NT	NT	NT	NT	−	NT	+	+
A	−	−	+	+	−	−	+	+	NT	NT	+	+
F	−	−	+	+	−	−	−	−	NT	NT	+	+
D	−	−	−	−	−	−	−	−	NT	NT	+	−
I	−	−	−	−	−	−	−	−	NT	NT	+	+
N	−	−	−	−	−	−	−	−	NT	NT	−	−

[a]Urines tested in strain TA98 in the presence of S9 and β-glucuronidase.

[b]Not tested.

was extracted from lung, liver and bladder according to the method of Gupta et al. [28]. DNA samples were analyzed for DNA adducts using two versions of the ^{32}P-postlabeling procedure [17,18] with minor modifications as reported previously [29].

Briefly, DNA samples (2.5–5.0 μg) were enzymatically digested to deoxyribonucleoside 3′-monophosphates with micrococcal endonuclease and spleen phosphodiesterase. Then the digests were either extracted with butanol or treated with nuclease P1. The mononucleotides were ^{32}P-postlabeled using 50 μCi of [γ-^{32}P]ATP by polynucleotide kinase-mediated phosphorylation. Thin-layer chromatography was used to separate the adducts. Areas of radioactivity were located by autoradiography, cut out, measured by scintillation counting, and the adduct levels quantitated.

RESULTS AND DISCUSSION

The quantitative results of our genotoxicity studies of these wastes and waste extracts have been published elsewhere [20,21,23]; the qualitative responses are summarized here. Table III summarizes the mutagenic responses in *Salmonella* of the first set of wastes and waste extracts, the mutagenic responses in *Salmonella* of the urine or urine extracts from rats gavaged with these wastes, and the responses of these wastes in the prophage-induction assay. Table IV summarizes the genotoxic responses of the second set of wastes in Salmonella and in the mammalian cell assays.

Liquid extraction, as opposed to solid-phase extraction, was the only extraction methodology that was suitable for these diverse wastes. However, as discussed previously [20], DCM may not be a suitable solvent for all types of wastes. Table III shows that DCM failed to extract mutagenic activity from 5 wastes (L, M, G, F, O) that were mutagenic in their crude, unextracted form. Nearly 80% of these wastes would have been detected as mutagenic if only the crude wastes had been tested. The additional time and expense required to prepare organic extracts of these wastes did

TABLE IV

GENOTOXIC RESPONSES OF WASTE EXTRACTS IN *SALMONELLA* AND MAMMALIAN CELLS

Waste extracts	Mutagenicity in *Salmonella*				Genotoxicity in mammalian cells			
	TA98		TA100		Mutagenicity in L5178Y/TK$^{+/-}$	Cytogenetic effects		Transformation
	+S9	−S9	+S9	−S9		SCEs	Chromosomal aberrations	
P	+	−	+	−	+	+	+	−
Q	+	−	−	−	+	−	−	−
R	+	−	−	−	+	+	−	−
S	−	−	−	−	+	+	−	−

not produce extracts that yielded much additional information that was not obtainable from the crude wastes.

However, not all wastes can be tested directly due to microbial contamination or physical state. For example, highly viscous wastes are difficult to pipette and dilute, complicating the generation of reproducible, quantitative results. Also, wastes with high or low pH may be highly toxic to cells and may have to be neutralized before bioassay. Thus, extraction/fractionation procedures will be necessary for some, if not most, hazardous wastes in order to examine their biological activity. An innovative approach involving fractionation by thin-layer chromatography coupled with the *Salmonella* assay has been shown to be useful with some wastes [4].

Judicious selection of a test matrix (and battery) is required in order to screen hazardous wastes in a cost-effective manner without an unacceptable loss of detection capability. For example, all of the wastes and waste extracts that were mutagenic in *Salmonella* in Table III would have been detected if they had been tested only in the presence of S9 (Table III), reducing the testing matrix in half. Considering the results with both strains (+/− S9) with the crude wastes and waste extracts, there were 9 mutagenic wastes identified, 7 of which were mutagenic in TA98+S9. Thus, if the crude wastes and extracts had been studied only in TA98 in the presence of S9, nearly 80% of the mutagenic wastes identified with the present matrix would have been identified.

Based on the wastes tested here, the urinary mutagenesis assay did not appear to be useful as a rapid screen to replace or complement testing the wastes or waste extracts directly in the *Salmonella* assay. Only 3 of the 10 wastes tested produced mutagenic raw urine, and all 3 of these wastes were mutagenic when tested directly in *Salmonella* (Table III). Extracting the urines by means of C_{18}/methanol elution did not identify a urine as mutagenic that was not identified as mutagenic from studies with raw urine (Table III). Considering the time and expense of performing the urinary mutagenesis assay, this assay was not a useful adjunct to testing the wastes or waste extracts directly for mutagenicity.

The prophage-induction assay in *E. coli* detected 5 crude waste samples that were not mutagenic in *Salmonella* (Table III). As described in the Introduction, the Microscreen prophage-induction assay has been shown to detect some carcinogenic metals and chlorinated organics and solvents that are not mutagenic in *Salmonella*. Metals and compounds of these types are present in most of the waste samples studied here [19], and the ability of some of these compounds to induce prophage may account for the detection by the prophage-induction assay of the 5 additional waste samples. Accumulating evidence indicates that prophage induction (and the SOS response in general) is a broader genetic end-point than reverse mutation in bacteria [30–32], making it especially useful for screening chemically diverse wastes.

A comparison of the genotoxic responses of DCM extracts of 4 wastes in the *Salmonella* assay to their responses in a set of mammalian cell assays indicates that the inclusion of mammalian cell assays may not have improved significantly the ability

to detect the genotoxicity of the wastes beyond that afforded by the *Salmonella* assay alone (Table IV). Waste S was the only waste detected by the mammalian cell assays that was not detected by the *Salmonella* assay. The BALB/c-3T3 transformation assay did not detect any of the extracts as positive, even though all 4 were genotoxic in one or more assays. Based on these limited results, it appears that this assay may not be useful for screening hazardous wastes.

Currently, there is only a small database on the use of mammalian cell assays with complex mixtures, let alone with hazardous wastes. Perhaps some of the reasons for this are that mammalian cell assays are difficult to use with toxic complex mixtures and are more costly and time-consuming to perform than microbial assays. Recently, two studies [33,34] have shown that mammalian cell assays may not provide much additional detection capability beyond that afforded by the *Salmonella* assay for pure compounds. Our results with these hazardous wastes suggest that the same may be true for complex mixtures.

The results of the hepatotoxicity study have been published [27] and are summarized in Table V. Based on histopathological evaluation of the liver, 8 of the 10 wastes were hepatotoxic (Table V). Under the experimental conditions, wastes H and O were non-hepatotoxic. Nine of the 10 wastes caused an increase in relative liver weight, and various wastes increased the serum concentrations of different combinations of the serum enzymes and bilirubin (Table V).

With histopathology as the criterion of hepatotoxicity, the best single predictor of

TABLE V

SUMMARY OF HEPATOTOXIC EFFECTS OF WASTES

Waste	n[a]	Dose (ml/kg)	Histopathology	Relative liver weight	Serum indicators of hepatic injury					
					AST	ALT	LDH	ALKPH	OCT	BILI
A	5	1	+[b]	+	−[c]	−	+	+	+	+
B	6	1	+	+	+	−	−	−	−	−
E	4	2.5	+	+	−	−	−	+	−	−
	2	5	+	+	+	+	−	+	−	+
G	5	5	+	+	−	−	−	−	−	+
H	6	5	−	+	−	−	−	−	−	−
J	6	0.5	+	+	+	+	−	−	+	−
K	6	0.5	+	+	+	+	−	+	+	−
L	4	2.5	+	+	+	−	+	−	−	−
M	5	5	+	+	+	+	+	+	−	−
O	6	5	−	−	−	−	−	−	−	−

[a] n = number of rats used for evaluation of hepatotoxicity.
[b] + = an increase compared to concurrent controls.
[c] − = no change compared to concurrent controls.

hepatotoxicity was relative liver weight [27]. Assessed individually, single serum indicators could not distinguish hepatotoxic from non-hepatotoxic wastes. The inability of any single serum indicator to identify correctly the hepatotoxic samples was due to false negatives rather than to false positives [27]. Therefore, the 5 commercially obtained serum indicators (AST, ALT, LDH, ALKPH, and BILI) were assessed collectively to determine how well they predicted hepatotoxicity as a battery. The serum battery was considered positive if at least one serum indicator was positive, and it was considered negative if all of the serum indicators were negative. As a battery, the serum indicators correctly identified the 8 hepatotoxic waste samples and the 2 non-hepatotoxic waste samples [27]. The advantages and disadvantages of the hepatic indicators for screening purposes have been discussed [35]; histopathologic evaluation was recommended rather than the serum battery in animal studies except when results are required before pathology results are available. The potential usefulness of the serum battery for monitoring human exposure to hazardous waste has been noted [35].

The chemical characterization available for these wastes (Table II) [19] is more extensive than would ordinarily be available for most complex wastes and allowed for a limited assessment of the relationship between chemical characterization and biological effects. Comparing observed toxicity to that expected based on a limited understanding of the chemical composition of the waste is important because one method used by the U.S. EPA to identify wastes as hazardous is based on partial chemical characterization [36]. As discussed extensively elsewhere [20,23,27], the observed biological effects (genotoxicity, cytotoxicity, hepatotoxicity) were not readily predicted from the chemical characterization data.

These results have important implications for assessing exposure to complex mixtures such as these hazardous wastes. As discussed previously, the biological activities of these mixtures are not readily predictable from knowledge of some of the chemicals present in the mixtures. Likewise, it is unlikely that monitoring individuals for exposure to one or a few chemicals will provide highly informative data regarding individual exposure to such complex mixtures. One possible approach to determining exposure to complex mixtures that contain a variety of mutagenic/carcinogenic compounds is the use of the ^{32}P-postlabeling technique described previously [17,18].

In order to examine the feasibility of this technique for exposure assessment, we have analyzed DNA from lung, liver and bladder of rats exposed to wastes M or L, which were mutagenic, caused DNA damage, were hepatotoxic, and produced mutagenic urine. One major DNA adduct was detected in rat bladder DNA following the oral administration of 2.5 ml/kg of waste L (Fig. 1). The relative adduct level was determined to be 3.4 adducts/10^9 nucleotides. DNA adducts were not detected in lung or liver DNA from the rat gavaged with waste L. No DNA adducts were detected in lung, liver or bladder DNA from the rat gavaged with waste M. It is interesting that waste L, which produced mutagenic urine that was relatively potent (259 revertants/ml of raw urine and 1586 revertants/ml equivalent of C_{18}/methanol con-

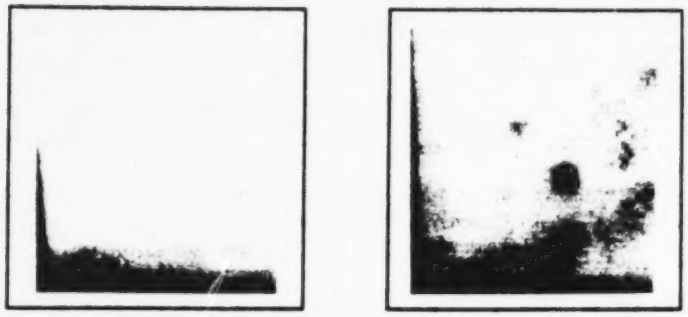

Fig. 1. Autoradiogram of PEI cellulose thin-layer chromatography fingerprint of a DNA adduct detected in rat bladder DNA 24 h after gavage by waste L. Autoradiogram was developed for 4 days at −80°C.

centrate) [20], also produced detectable DNA adducts in the bladder. Apparently, mutagens in the urine were able to bind covalently to bladder tissue DNA.

One main purpose in these studies has been to determine which individual bioassay or groups of bioassays could serve as inexpensive, rapid screening tools to assess the toxicity of a large number of chemically different industrial wastes. The limited number of hazardous wastes that we have studied here cannot be considered to represent the 'universe' of wastes. In addition, there are many other bioassays that we have not yet examined that may be useful for screening hazardous wastes. Given these limitations, however, our studies suggest that the *Salmonella* assay using strain TA98 in the presence of S9 or the prophage-induction assay in the presence of S9 may be useful in screening wastes for genotoxicity. Based on the available limited data, relative liver weight and a battery of serum indicators appeared potentially useful for routine screening of complex mixtures for hepatotoxicity.

The combination of this hepatotoxicity assay with one of the genotoxicity assays might provide a cost-effective, rapid and simple battery of bioassays that could be used routinely to characterize large numbers of waste samples. The possible role of ^{32}P-postlabeling for determining adducts resulting from exposure to hazardous wastes should be explored further in order to determine the usefulness of this approach for assessing exposure.

Both government [37–39] and industry [2,40] have recognized the important role that short-term tests could play in the toxicological assessment of hazardous wastes. We have reported here on the use of only genotoxicity and hepatotoxicity bioassays to evaluate hazardous wastes; however, wastes may induce other biological effects, such as neurotoxicity. Additional investigations are needed to explore the effects of hazardous wastes at these and other toxicological end-points. The results presented here suggest that short-term bioassays may be useful adjuncts to chemical analysis

in identifying wastes as hazardous. They also illustrate the importance of developing means of assessing exposure to complex mixtures rather than to single chemicals.

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